constructed by sandwiching a polymer spacer between two infrared transparent windows, creating a closed channel system. The channels in the spacer were laser cut to establish the layout of the mixer. The mixer takes advantage of hydrodynamic focusing with two side flow channels, squeezing a middle sample channel into a thin jet and initiating fast mixing through diffusion and advection. The mixing region is probed with a laser source in the mid-infrared region, then magnified and imaged on a focal plane array detector for absorption measurements. The mixer was experimentally calibrated in order to determine the amount of time per pixel in the detector. A pD jump mixing experiment of an adenosine monophosphate solution was employed in order to establish a mixing time on the order of 350 μs. Finally, a flow study with H2O and D2O was completed in order to compare experimental results with simulation. With the established mixing time and use of vibrational spectroscopy, this system can be applied to the study of protein and enzymatic reactions.

3097-Pos Board B789

Ultra Fast Raman Hyperspectral Imaging using Bragg Tunable Filters and a High Performance Emccd Camera

Félix Thouin[1], Frédéric Leblond[2], Richard Martel[1], Marc Verhaegen[3], Olivier Daigle[4].
[1]Departement of chemistry, University of Montreal, Montreal, QC, Canada, [2]Departement of engineering physics, École Polytechnique de Montréal, Montreal, QC, Canada, [3]Photon Etc., Montreal, QC, Canada, [4]Nüvü Caméras, Montreal, QC, Canada.

Because of its high specificity to a variety of molecular processes and its low sensitivity to the presence of water, Raman hyperspectral imaging is regarded as a very promising technique to help pathologists improve the accuracy of medical diagnostics when compared to conventional histopathological analysis. However, since on average approximately one photon per million undergoes Raman scattering, acquisition time per hyperspectral image is very long, typically of about 6 hours. This significantly reduces the appeal of this technique for ex-vivo diagnostics and makes in-vivo applications impracticable. To increase acquisition speed, a Raman hyperspectral imager based on holographic Bragg tunable filters was used and images of carbon nanotubes could be acquired 30 times faster than with a conventional confocal microscope optimised for fast mapping. This speed gain over traditional methods was further enhanced when also using a low-noise EMCCD camera, resulting in measurements performed 150 times faster. These results thus indicate that in vivo and ex vivo applications of wide-field Raman hyperspectral imaging are now at reach, hence paving the way for real time tumor detection during surgery.

3098-Pos Board B790

Difference FT-IR Studies on the Effects of Buffers on Nucleotide Binding to Reca

Joshua Temple, Gina MacDonald, Dr.
Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive, MSC 4501, Harrisonburg, VA 22801, USA.

The *Escherichia coli* protein RecA catalyzes DNA strand exchange and plays a role DNA repair and genetic recombination. Nucleotide binding influences RecA oligomerization and its affinity for DNA. Previous studies in our lab have shown buffer-specific changes in RecA stability and unfolding transitions. Past circular dichroism (CD), infrared (IR), and fluorescence studies suggest only minimal buffer dependent changes in nucleotide binding and secondary structure that did not explain the large buffer dependent differences in RecA stability and unfolding profiles. These observations led to further investigations of how the four common biological buffers Tris, HEPES, MES, and PO_4 alter RecA structure and nucleotide binding. Here we have employed difference infrared spectroscopy utilized in conjunction with caged nucleotides to generate RecA-ADP *minus* RecA difference infrared spectra in each of the four buffers. These higher resolution studies are aimed at detecting if the buffers alter nucleotide binding to RecA. Preliminary results show that ADP binding results in perturbations in Gln, Glu, Asp, Asn, Tyr, and Lys residues and secondary structural changes. Initial comparisons of difference spectra obtained in the four buffers show some similar changes but also show some differences. These differences between RecA-ADP *minus* RecA difference spectra will be discussed.

3099-Pos Board B791

FT-IR Spectroscopy and Density Functional Theory Calculations of Carbon-13 Isotopologues of the Helical Peptide Z-AIB(6)-OTBU

Matthew A. Kubasik, Timothy Zeko, Steven F. Hannigan, Timothy Jacisin.
Fairfield University, Fairfield, CT, USA.

Isotope-edited FT-IR spectroscopy is a combined synthetic and spectroscopic method used to characterize local (e.g., residue-level) vibrational environments of biomolecules. We have prepared the 3_{10} helical peptide Z-Aib_6-OtBu and seven ^{13}C-enriched analogues which vary only in the number and position(s) of ^{13}C=O isotopic enrichment. FT-IR spectra of these eight peptides solvated in the nonpolar aprotic solvent dichloromethane have been collected and compared to frequency, intensity, and normal mode results of DFT calculations. Single ^{13}C enrichment of amide functional groups tends to localize Amide I vibrational eigenmodes, providing residue-specific information regarding the local environment (e.g., hydrogen bonding or solvent exposure) of the peptide bond. Double ^{13}C enrichment of Z-Aib_6-OtBu allows for examination of inter-amide coupling between two labeled amide functional groups, providing experimental evidence of inter-amide coupling in the context of 3_{10} helical structure. Although the calculated and observed inter-amide couplings of Z-Aib_6-OtBu are a few cm^{-1} and less, the eight peptides exhibit distinct infrared spectra, revealing details of inter-amide coupling and residue level vibrational environments.

3100-Pos Board B792

Ulrtrafast Water Dynamics in Bacteriorhodopsin

Philipp Alt, Miriam Colindres-Rojas, **Rolf Diller**.
Physics, University of Kaiserslautern, Kaiserslautern, Germany.

Protein bound water molecules play an important role for protein function. In the trans-membrane proton pump bacteriorhodopsin (BR) water molecules in the proton transport channel and in the retinal chromophor binding pocket have been shown by means of infrared vibrational spectroscopy to participate in the light driven proton transport mechanism (Garczarek et al., Nature 2006, 439, 109). Static low temperature FTIR experiments strongly suggest that the pentagonal water cluster involving three water molecules W401, W402 and W406 as well as the retinal Schiff-base, Asp85 and Asp212, is perturbed when the ground state BR570 is photoconverted to the K610 state (H. Kandori et al., J.Phys.Chem.B 1998, 102, 7899). We have now investigated the spectral range above 3.3 μm by means of femtosecond time resolved transient mid-IR spectroscopy. Here we find a broad IR absorption band which bleaches within (or even faster than) retinal isomerization, i.e. 0.5 ps. This finding is strongly corroborated by quantum-chemical QM/MM calculations that attribute this continuum band to a polarization coupling between the protonated retinal Schiff-base N-H stretch and water W402 in BR570 (M. Baer et al., ChemPhysChem 2008, 9, 2703). Our results indicate that the pentagonal water cluster is heavily perturbed already on this time scale.

3101-Pos Board B793

A New Method for Analysis of Temperature Dependent IR Amide I Spectra of Peptides and Proteins

Jan Kubelka.
Department of Chemistry, University of Wyoming, Laramie, WY, USA.

Changes in the amide I' IR band with temperature are widely used for studies of structural transitions in peptides and proteins. Since amide I' exhibits inherent temperature dependent frequency shifts, standard mixture analysis methods are not applicable. To reliably extract the true thermodynamic states, frequency shifts of the component spectra must be explicitly taken into account. New methods termed Shifted Multivariate Spectra Analysis (SMSA) and parametric SMSA (pSMSA) were developed. SMSA uses no specific functional form for the transition (soft modeling), while the parametric variant (pSMSA) assumes a thermodynamic model (hard modeling). The implementation is optimized specifically for amide I' IR in that it takes advantage of known, linear dependence of the frequencies, as well as intensities, on temperature. The methods are first tested on sets of synthetic data with varying amounts of noise as well as on a real experimental amide I' data for the thermal unfolding of an α-helical peptide. The synthetic data tests demonstrate that the methods very reliably recover the correct parameters, although the non-parametric SMSA is subject to the rotational ambiguity. Application to the peptide experimental amide I' data illustrates additional complications encountered with the analysis of real systems, namely the correction for the side-chain spectral bands and interference of spectral shape changes. Finally the pSMSA is applied to the analysis of site-specific thermal unfolding of two small α-helical proteins from sets of multiple ^{13}C isotopically edited amide I' spectra.

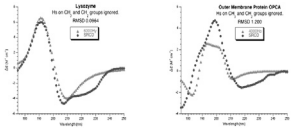
3102-Pos Board B794

Computing Theoretical Circular Dichroism of Proteins using the Dipole Interaction Model (DINAMO) with a United Atom Approach

Rahul Nori[1], Igor V. Uporov[2], Neville Y. Forlemu[3], Yvonne E. Bongfen[4], Tsvetan Aleksandrov[1], Kathryn A. Thomasson[1].
[1]University of North Dakota, Grand Forks, ND, USA, [2]Lomonosov Moscow State University, Moscow, Russian Federation, [3]Georgia Gwinnett College, Lawrenceville, GA, USA, [4]Oklahoma Baptist University, Shawnee, OK, USA.

The dipole interaction model is a classical electromagnetic theory for calculating the π-π* transitions of peptides and proteins. DInaMo reduces all amide

chromophores to single points with anisotropic polarizability and all nonchromophoric aliphatic atoms to points with isotropic polarizability; all other atoms are ignored. By determining interactions among the chromophoric and nonchromphoric parts of the molecule using empirically derived polarizabilities, the rotational and dipole strengths are determined leading to the calculation of the CD spectrum for each molecule. DInaMo software is being modified to reduce computational demand by collapsing hydrogens into the atoms to which they are bound (i.e., united atom). Crystal structures of proteins containing less than 300 residues are energy minimized using NAMD. Hydrogens attached to various groups (e.g., CH_3, CH_2, or CH groups) are then deleted programmatically. Deleted hydrogen polarizabilities are either ignored or added to the atom to which they are bound. Theoretically predicted CD for a variety of proteins (26 different structures, examples pictured below) are compared with synchrotron radiation CD data. Theory agrees with experiment showing bands with similar morphology and absorption maxima for the π-π* transitions.



Bioengineering

3103-Pos Board B795

Photoregulation of Small G Protein Kras using Photochromic Molecules

Seigo Iwata, Shinsaku Maruta.
Soka Univ, tokyo, Japan.

Ras is one of small G-proteins known as a molecular switch mediating cellular signalling. Switching ON state of Ras is induced by exchange of bound GDP for GTP and OFF state is by hydrolysis of GTP to GDP. Interestingly, the core nucleotide-binding motif of Ras is considerably conserved with the ATP driven motor proteins, myosin and kinesin. Therefore, it is believed that these bio-molecular machines share common molecular mechanism utilizing nucleotide hydrolysis cycle. Previously, we have incorporated photochromic molecules, 4-phenylazophenyl maleimide (PAM), into the functional site of kinesin as a photo-switching device and succeeded to regulate kinesin ATPase activities reversibly upon visible light (VIS) and ultra-violet (UV) light irradiation. Therefore, it is expected that Ras can be also regulated using photochromic molecules.

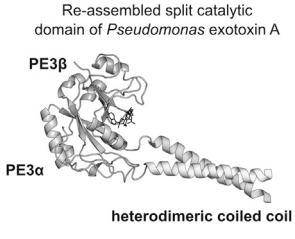
In this study, we performed basic study to control the function of Ras reversibly using photochromic molecules upon VIS and UV light irradiations. First, in order to monitor the exchange of bound GDP for GTP, we synthesized a new fluorescent GTP analogue, NBD-GTP and NBD-GDP, which change their fluorescent intensity during their binding to Ras. Second, we synthesized a new photochromic molecule, iodoacetyl spiropyran (IASP) and iodoacetyl fulgimide (IAFI) that are incorporated into cysteine residue specifically. And the GTPase activity of Ras was monitored by the quantitative analysis of GTP and GDP in the active site of Ras using reverse phase column chromatography on HPLC. We have designed three kinds of Ras mutants Y32C, I36C, and Y64C. The mutants were prepared using *E.coli.*expression system and modified with PAM and IASP stoichiometrically. It was suggested that the GTPase activities of the Ras mutants modified with PAM were reversibly alternated upon VIS and UV light irradiations. The Ras mutants modified with IASP and IAFI were also examined.

3104-Pos Board B796

Structural Complementation of the Catalytic Domain of Pseudomonas Exotoxin A

Erin L. Boland[1], Crystal M. Van Dyken[1], Rachel M. Duckett[1], Andrew J. McCluskey[2], **Gregory M. Poon**[1].
[1]Pharmaceutical Sciecnes, Washington State University, Pullman, WA, USA, [2]Microbiology and Immunobiology, Harvard Medical School, Boston, MA, USA.

Pseudomonas exotoxin A (PE3) is a specific ADP-ribosyltransferase for eukaryotic elongation factor 2 (eEF2). ADP-ribosylation of eEF2 arrests ribosomal protein synthesis and potently induces cell death by apoptosis. A single molecule of functional homologs of exotoxin A, such as Diphtheria toxin, is known to be sufficient to kill a cell. We have designed and characterized catalytically inactive fragments of PE3 that are capable of structural complementation. We dissected PE3 at an extended loop and fused each fragment to one subunit of a heterospecific coiled coil.*In vitro* ADP-ribosylation and protein translation assays demonstrate that the resulting fusions - supplied exogenously as genetic elements or purified protein fragments - had no significant catalytic activity or effect on protein synthesis individually, but in combination catalyzed the ADP-ribosylation of eEF2 and inhibited protein synthesis. Although complementing PE3 fragments are less efficient catalytically than intact PE3 in cell-free systems, co-expression in live cells transfected with transgenes encoding the toxin fusions inhibits protein synthesis and causes cell death comparably as intact PE3. **Split PE3 offers a direct extension of the immunotoxin approach to generate bispecific agents that may be useful to target complex phenotypes.**



3105-Pos Board B797

Fibrin Fibers: Blocking the B:B Knob-Pocket Interaction

Stephen Baker[1], Ashley Carson-Brown[2], Martin Guthold[3], Thomas Barker[2].
[1]Wake Forest University, Winston Salem, NC, USA, [2]Wallace H. Coulter Dept. of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA, [3]Physics, Wake Forest University, Winston Salem, NC, USA.

Fibrin clot formation has been studied to determine the mechanical properties of fibrin fibers modified by blocking the B-b knob-pocket interaction. Synthetic B-knob peptides AHRPYAAC or AHRPYAAC-Peg have been added to a fibrinogen solution to allow for binding to the b-pockets prior to clot formation. After fibrin clot formation, a combined atomic force microscopic (AFM)/optical microscopic technique was used to study the properties of individual fibrin fibers in buffer. Mechanical testing of fibers was done using the AFM to laterally stretch individual fibers suspended over 13.5μm wide groves in a transparent substrate. The optical microscope, located below the sample, was used to monitor the stretching process. We found that the density and lateral aggregation of fibers was hindered by blocking the b-pockets with the synthetic B-knobs.

3106-Pos Board B798

Principles for the Rational Design of Allosterically Cooperative Biomolecular Receptors

Anna J. Simon[1], Alexis Vallée-Bélisle[2], Francesco Ricci[3], Herschel M. Watkins[1], Kevin W. Plaxco[4,5].
[1]Biomolecular Science and Engineering Program, University of California, Santa Barbara, Santa Barbara, CA, USA, [2]Département de Chimie,, Université de Montréal, Montréal, QC, Canada, [3]Dipartimento di Scienze e Tecnologie Chimiche, University of Rome, Tor Vergata,, Rome, Italy, [4]Biomolecular Science and Engineering Program, UC Santa Barbara, Santa Barbara, CA, USA, [5]Biochemistry and Chemistry, University of California, Santa Barbara, Santa Barbara, CA, USA.

Allosteric cooperativity is used ubiquitously throughout nature to generate steeper, more responsive input-output behaviors. The focus of my work has been to rationally introduce this valuable property into several classes of normally non-cooperative biomolecular receptors. My approach to this end employs the classical population-shift (between "tense" and "relaxed" states) mechanism underlying the cooperative oxygen binding of hemoglobin. Specifically, I have designed receptors that equilibrate between two conformations, the more stable of which binds the target ligand only weakly and the less stable of which exposes two high-affinity ligand binding sites. The binding of the first copy of the ligand shifts this conformational equilibrium towards the later, higher-affinity state, improving the affinity of the second binding event and thus producing a steeper, highly cooperative response. One approach to this end involves receptors that equilibrate between an intrinsically disordered conformation and a folded conformation containing two target-binding sites. The folding of the entire receptor upon binding the first copy of target molecule improves the affinity of the second, leading to cooperative binding. The observed cooperativity of my redesigned receptors is quantitatively dependent on the equilibrium constant K_S, describing the switch between the tense and relaxed (e.g., folded and unfolded) states. Effectively maximal sensitivity (i.e., a Hill coefficient within error of the theoretically expected 2 and a 9-fold increase in sensitivity) is achieved as $K_S$falls below 0.0025. The enhanced responsiveness of these cooperative receptors should improve their utility in applications, such as biosensors, biomolecular logic gates, and "smart," responsive biomaterials, in which steeper, more sensitive input−output behavior is of value.

3107-Pos Board B799

Necking and Failure of Constrained Contractile 3D Microtissues Induced by Cell Derived Tension

Vivek B. Shenoy[1], Hailong Wang[1], Alexander A. Svoronos[2], Thomas Boudou[1], Jeffrey R. Morgan[2], Christopher S. Chen[1].
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]Brown University, Providence, RI, USA.

We report a fundamental morphological instability of constrained 3D microtissues induced by a positive chemomechanical feedback between actomyosin-driven contraction and the mechanical stresses arising from the constraints. Using a 3D model for mechanotransduction we find that perturbations in the